AMENDMENTS TO THE SPECIFICATION

On pages 2-3, please amend the paragraph bridging pages 2-3 as follows:

The immobilization of biological polymers on solid supports has also had significant impact on drug discovery and medical diagnostic methods. One important invention in these fields is described in U.S. Pat. No. 5,143,854 and in Application Ser. Nos. 624,120, filed Dec. 6, 1990, now abandoned, ~~and 805,727, filed Dec. 6, 1991,~~ U.S. Pat. No. 5,424,186, and in PCT patent publication No. 90/15070 to Pirrung et al., each of which is incorporated herein by reference. In brief, the invention provides methods and reagents for synthesizing very large numbers of different compounds, particularly biological polymers, in distinct and spatially-addressable locations in a very small area on a substrate. Another description of the invention is found in Fodor et al., 15 Feb. 1991, Science 251:767-773, and the integration of the invention with other drug discovery methods is described in Dower and Fodor, 1991, Ann. Rep. Med. Chem. 26:271-280. A related method uses a photoactivatable derivative of biotin as the agent for immobilizing a biological polymer of interest onto a solid support; see U.S. Pat. No. 5,252,743, and PCT patent publication No. 91/07087 to Barrett et al., each of which is incorporated herein by reference.

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On page 3, please amend the first full paragraph as follows:

Recent approaches to genetic analysis are increasingly placing importance on performing parallel hybridizations in an array format. Applications of the parallel hybridization format include generating diagnostic arrays for tissue typing or diagnosis of genetic disorders (see PCT patent publication No. 89/11548, incorporated herein by reference), DNA sequencing by hybridization, DNA fingerprinting, and genetic mapping (see U.S. patent application Ser. Nos. 624,114, now abandoned, and ~~626,730, filed Dec. 6, 1990~~ U.S. Patent No. 5,547,839, each of which is incorporated herein by reference; see also Khrapko et al., 1991, J. DNA, Seq. Map. 375-388). In these applications of probe arrays, the information content of the array increases as the number of probes is increased. The size limit of the array is dictated by the ability to automate and miniaturize the fabrication of the array.

a2

On page 4, please amend the last full paragraph as follows:

a3

In certain embodiments, the solid support is an array ~~comprises~~ comprising a library of target moieties.

On page 5, please amend the first full paragraph as follows:

a4

An aspect of the invention provides for a method of ~~inking~~ linking a probe to a solid support comprising providing a solid support having an array of surface-bound oligonucleotides, hybridizing to a surface-bound oligonucleotide a probe comprising a pairing oligonucleotide sequence complementary to the surface-bound oligonucleotide sequence and a target moiety, and forming a covalent bond between the pairing oligonucleotide sequence and either the surface-bound oligonucleotide or the solid support.

Amt note

On page 8, please amend the ~~second~~ third full paragraph as follows:

a5

It will be apparent to those skilled in the art that the methods and ~~compositins~~ compositions of the present invention will find application in any of the above-noted processes for solid phase synthesis of biological polymers and other small molecule ligands.

On page 14, please amend the third full paragraph as follows:

a6

A "target moiety" is a solvated molecule or particle that is recognized by a particular molecule. Examples of target moieties that can be investigated with the present invention include, but are not restricted to, agonists and antagonists for cell membrane receptors, toxins and venoms, viral epitopes, hormones, opiates, steroids, hormone receptors, peptides, enzymes, enzyme substrates, cofactors, drugs, lectins, sugars, oligonucleotides, oligosaccharides, proteins, and monoclonal antibodies. Target moieties include particles with surfaces that expose the ~~complementar~~ complementary nucleic acid base sequence required for it to become attached.

On page 21, please amend the first full paragraph as follows:

In one embodiment, silicon surfaces were exposed to solution mixtures of dodecyl trichlorosilane and trichloroacetyl (TFA) or trifluoroacetyl (TCA) undecyl trichlorosilane (TCS) to produce SAM with mixed surface compositions. Contrary to the monolayer coverage obtained using alkanethiol SAMs, the thickness of mixed TCS SAMs varied with immersion times. For ~~immersions~~ immersion times lower than 1 hour the SAM thickness was approximately 25-30 Å, corresponding to a monolayer level surface coverage. However, as immersion times increased the thickness increased, until at 8 hour immersion the adlayer thickness was above 100 Å , in the multiplayer film regime. TCS may be much more reactive then the triethoxy or trimethoxy silanes and thus more extensive crosslinking between silane chains occur, resulting in gel-like structures over longer reaction times. Therefore the reaction times for TCS were limited to 30 to 60 minutes to prevent multilayer formation. In general the kinetics of TFA TCS SAM formation were more rapid compared to the TCA TCS SAMs, possibly due to the lower chemical stability of the TFA protecting group, which is more readily removed by trace amounts of residual water to prematurely expose the hydroxy group, resulting in unwanted crosslinking side reactions. In one embodiment, the appropriate functional group, e.g. hydroxy, is not generated in the first monolayer.

On pages 21-22, please amend the paragraph bridging pages 21-22 as follows:

In one embodiment, mixed methyl and hydroxy terminated TCS formed SAMs with varied hydroxy group densities. However, since the TCS group is highly reactive towards any hydroxy groups TCA and TFA protecting groups can be used to prevent side reactions from occurring. Clean silicon surfaces were immersed in xylene solutions containing mixtures of octyl and TFA or TCA TCS species, at a concentration of 1μl/ml for 1 hour, under ambient conditions. Although TCS is very hygroscopic, SAM formed readily in ambient air as long as the solvent was dry. The reaction mechanism was also hydrolysis of the TCS groups to silanol groups, possibly with residual water adsorbed on the silicon surfaces as well as traces in the organic solvent. The silanols would then quickly react with hydroxy groups on the native oxide surfaces, produced in the reducing environment of the base bath used to clean the silicon chips. Subsequent immersion in a basic solution would remove the protecting groups to expose the

a8 corrected

hydroxy groups. Both TFA and TCA TCS species can produce the hydroxy surface groups, but the TFA protecting group is less stable compared to TCA groups.

On pages 22-23, please amend the paragraph bridging pages 22-23 as follows:

a9

Oligonucleotides of the present invention may include non-phosphate internucleosidic linkages. Many such linkages are known in the art, e.g., phosphorothioate: Zon and Geiser, Anti-Cancer Drug Design, 6:539-568 (1991); Stec et al., U.S. Pat. No. 5,151,510; Hirschbein, U.S. Pat. No. 5,166,387; Bergot, U.S. Pat. No. 5,183,885; phosphorodithioates: Marshall et al., Science, 259:1564-1570 (1993); Caruthers and Nielsen, International application PCT/US89/02293; phosphoramidates, e.g., -OP(=O)(NR_1 R_2)-O- with R_1 and R_2 hydrogen or C_1-C_3 alkyl; Jager et al., Biochemistry, 27:7237-7246 (1988); Froehler et al., International application PCT/US90/03138; peptide nucleic acids: Nielsen et al., Anti-Cancer Drug Design, 8:53-63 (1993), International application PCT/EP92/01220; methylphosphonates: Miller et al., U.S. Pat. No. 4,507,433, Ts'o et al., U.S. Pat. No. 4,469,863; Miller et al., U.S. Pat. 4,757,055; and P-chiral linkages of various types, especially phosphorothioates, Stec et al., European ~~patent application 506,242 (1992)~~ Patent No. EP0506242, and Lesnikowski, Bioorganic Chemistry, 21:127-155 (1993). Additional non-phosphate linkages include phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoranilidate, alkylphosphotriester such as methyl- and ethylphosphotriester, carbonates such as carboxymethyl ester, carbamate, morpholino carbamate, 3'-thioformacetal, silyl such as dialkyl (C_1-C_6)- or diphenylsilyl, sulfamate ester, and the like. Such linkages and methods for introducing them into oligonucleotides are described in many references, e.g., reviewed generally by Peyman and Ulmann, Chemical Reviews 90:543-584 (1990); Milligan et al., J. Med. Chem., 36:1923-1937 (1993); Matteucci et al., International application PCT/US91/06855.

On page 30, please amend the third full paragraph as follows:

a10

In one embodiment, the oligonucleotide surface density is controlled by manipulating the composition and chemical ~~moeties~~ moieties of the silane SAM formed on the silicon surface.

On pages 30-31, please amend the paragraph bridging pages 30-31 as follows:

Oligonucleotide chips stored dry at 4°C for over three months did not demonstrate any significant decrease in the amount of DNA hybridized to the surface, indicating that the oligonucleotide chips are ~~chemical~~ chemically stable and can be stored for long periods of time prior to use. Tests on the recycle ability of the oligonucleotide surfaces were performed by heating an oligo chip with ^{32}P-labeled complementary oligonucleotide hybridized onto the surface, to 90° C in 0.1wt % SDS aqueous solution for 30 minutes, to remove the ^{32}P-labeled oligonucleotide chains. Then the oligo chip was rinsed with deionized water and washed again in 90° C 0.1 wt% SDS aqueous solution for 30 minutes, after which only background radiation was detected, indicating complete removal of the ^{32}P-labeled oligo from the surface. Next, the oligonucleotide chip was immersed in a fresh solution of complementary, radiolabeled oligonucleotide chains, and the amount of oligo hybridized to the oligonucleotide chip was measured by liquid scintillation counting. For all cycles complete desorption of labeled oligonucleotide was observed after heating, and there was no decrease in ^{32}P radiation signal during the subsequent hybridization reactions. This is expected since the oligonucleotide surfaces are covalently bound to the silicon substrate, and the nucleotide bases are chemically stable in the absence of enzymes. Further, the results demonstrate the thermal stability and recycle capabilities of the surface immobilized oligo chips.

On page 31, please amend the first full paragraph as follows:

In one embodiment, the ability to produce oligonucleotide chips on silicon surfaces with high sequence fidelity, thermal stability and with consistent, controllable surface density may be the basis for the synthesis of oligonucleotide chips with optimal hybridization performance, to immobilize oligonucleotide or DNA chains to a flat silicon surface. The oligonucleotide surfaces themselves could be used as probes to detect complementary oligonucleotides that are ssDNA in solution, or as the platform to immobilize a longer chain dsDNA to form a surface probe using ssDNA.

On page 32, please amend the first full paragraph as follows:

The target moiety is a molecule which may be contacted with a test sample in order to determine if an interaction is occurring between the target moiety and a molecule in the test sample. Examples of target moieties that may be used in accord with the invention include, but are not restricted to, agonists and antagonists for cell membrane receptors, toxins and venoms, viral epitopes, hormones, opiates, steroids, hormone receptors, peptides, enzymes, enzyme substrates, cofactors, drugs, lectins, sugars, oligonucleotides, oligosaccharides, proteins, peptides, antibodies, antibody fragments and small molecules. Oligonucleotide target moieties may be RNA or DNA, may be single stranded, double ~~standed~~ stranded or triple ~~standed~~ stranded and may be linear or contain elements of secondary and/or tertiary structure. In a preferred embodiment, the target moiety is a double stranded nucleic acid molecule which may be converted into a single stranded oligonucleotide via separation of the two strands. A nucleic acid targeting moiety may be of any length, but preferable comprises from 5 to 5,000 nucleotides, more preferably from 20 to 2,000 nucleotides and most preferably from 50 to 1,000 nucleotides.

a13

On pages 37-38, please amend the paragraph bridging pages 37-38 as follows:

The conjugate may also comprise a crosslinking reagent attached to the pairing oligonucleotide which can covalently attach the pairing oligonucleotide to the capture oligonucleotide. Any crosslinking agent capable of covalently attaching two oligonucleotides may be used. Various descriptions of suitable crosslinking agents are described ~~in~~ above. In a preferred embodiment, the crosslinking agent is psoralen. Psoralen is a photoactivated crosslinking molecule with a rigid, flat structure that readily intercalates within the dsDNA double helix, preferable between an AT sequence. Both the furan and pyrone functional groups of the psoralen compound are photolyzed with long wavelength UV light (365 nm) to form covalent bonds with particular nucleotide bases. The furan side is 4 times more reactive than the pyrone side an overwhelmingly favors reacting with T nucleotides, though they have limited reactivity also with C and U nucleotides. The crosslinking can result in the formation of monoadducts or diadducts by varying the wavelength and intensity of the UV illumination source. Finally, the crosslinking is reversible by exposure to short wavelength (254 nm) UV

a14

light, which appears to cleave the covalent bonds. Psoralen and methylated psoralen derivatives have been used to crosslink two complementary ssDNA strands of a dsDNA duplex together. Psoralen, psoralen derivatives and special phosphoramidites with 5' psoralen linkers are commercially available (Glen Research).

On page 39, please amend the first full paragraph as follows:

The described conjugates may be used in conjunction with the immobilized capture oligonucleotides to produce an array of targeting moieties. The targeting moieties are immobilized onto the array via hybridization between the capture oligonucleotide and the pairing oligonucleotide of the conjugate. In certain embodiments the capture and pairing oligonucleotides are ~~covalent~~ covalently attached using a crosslinking agent. The array preferably comprises a variety of targeting moieties at known, or addressed, locations. The array may then be contacted with a test sample under conditions which promote interaction between molecules in the test sample with one or more of the targeting moieties. The identities of the targeting moieties capable of interacting with a component of the test sample may be determined based on detecting the interaction of the molecules at a particular location on the array.

On page 48, please amend the second full paragraph as follows:

The above-mentioned immobilization technique covalently attaches dsDNA or ssDNA sequences to silica surfaces via their 5' terminus. Careful adjustment of the surface oligo density, dsDNA solution concentration and UV photolysis conditions are to control the dsDNA surface ~~surface~~ density. Furthermore, the underlying oligo surface prevents nonspecific adsorption of the dsDNA or ssDNA chain. This system has many advantages over existing methods. The *in situ* oligo synthesis combined with hybridization to dangling-end dsDNA is a straightforward approach that produces dsDNA and ssDNA surface arrays with controlled surface densities for a wide range of possible ~~applicatons~~ applications. It does not require chemical crosslinking or enzymatic reactions, which reduce the system consistency and reproducibility and complicate the manufacturing process. Using dsDNA PCR product

decreases the production time and high costs associated with the computer-aided design and microlithography techniques required to produce oligo microarrays.

On page 50, please amend the first full paragraph as follows:

Standard phosphoramidite synthesis chemistry is employed with the derivatized silicon surfaces as the substrate/support.~~: Insert hydroxyl~~ Hydroxyl terminated silane derivatized silicon chips are inserted into empty synthesis columns. The coupling reaction time is over 75 seconds to ensure optimal coupling efficiency, and remove the 5' DMT protecting group to expose a 5' hydroxy group. For the bases adenine (A), guanine(G), and cytosine(C), use Ultramild® phosphoramidites for mild deprotection. Wash the chips in the column with acetonitrile for 60 seconds, then dry with nitrogen gas for 60 seconds. Wash chips with acetone followed by 95% ethanol, and dry with nitrogen stream. Immerse the chips in 0.05M potassium carbonate in dry methanol for 2 hours, followed by 95% ethanol rinse and dried with nitrogen stream to remove the base protecting groups~~,~~. To remove the cyanoethyl groups immerse chips in 20 v/v% piperidine in dimethyl formamide for 2 hours, followed by 95% ethanol rinse and dried with nitrogen stream. Coupling efficiency increases with decreasing density of hydroxy reactive sites on the derivatized silicon surfaces, with 99+% efficiency when density is lower than $5x10^{-11}$ moles/cm^2 .

On pages 50-51, please amend the paragraph bridging pages 50-51 as follows:

Standard phosphoramidite synthesis chemistry is employed with the derivatized silicon surfaces as the substrate/support.~~: Insert hydroxyl~~ Hydroxyl terminated silane derivatized silicon chips are inserted into empty synthesis columns. Add on a C2 psoralen phosphoramidite, with coupling time adjusted to 900 seconds to maximize coupling efficiency and remove the 5' DMT protecting group to expose a 5' hydroxy group. For the bases adenine (A), guanine(G), and cytosine(C), use Ultramild® phosphoramidites for mild deprotection. Wash the chips in the column with acetonitrile for 60 seconds, then dry with nitrogen gas for 60 seconds. Wash chips with acetone followed by 95% ethanol, and dry with nitrogen stream. Immerse the chips in 0.05M potassium carbonate in dry methanol for 2 hours, followed by 95% ethanol rinse and

dried with nitrogen stream to remove the base protecting groups,. To remove the cyanoethyl groups immerse chips in 20 v/v% piperidine in dimethyl formamide for 2 hours, followed by 95% ethanol rinse and dried with nitrogen stream. Coupling efficiency increases with decreasing density of hydroxy reactive sites on the derivatized silicon surfaces, with 99+% efficiency when density is lower than $5x10^{-11}$ moles/cm^2.